

COOLING APPARATUS USED FOR CRYONIC PRESERVATION,  
AND CORRESPONDING OPERATING METHOD

The invention relates to a cooling equipment, especially for the cryopreservation of biological samples, in accordance with the preamble of Claim 1 as well as to a  
5 corresponding operating method according to the preamble of Claim 23.

The freezing of biological samples such as, e.g., stem cells in order to preserve their vitality is known within the scope of so-called cryopreservation. A cooling down to less than  $-130^{\circ}\text{C}$  is necessary here for a complete preservation of vitality so that liquid nitrogen is usually used as cooling agent. However, not only the low storage  
10 temperature is important for the preservation of vitality but also the observance of a given temperature course in time during freezing and thawing.

In order to meet these requirements, cooling equipment is obtainable, e.g., from DE 88 07 267.3 that uses liquid nitrogen with a boiling point of  $-196^{\circ}\text{C}$  as cooling agent. The liquid nitrogen is at first located in a cooling agent storage container and is  
15 heated in it by an electrically operated evaporator, the outgassing nitrogen being conducted via a cooling agent supply line into a cooling chamber and correspondingly cools its inner space so that material to be cooled located in the cooling chamber is frozen.

However, the mere outgassing of nitrogen by the evaporator only makes cooling  
20 agent temperatures near the boiling point of  $-196^{\circ}\text{C}$  possible, whereas, on the other hand, the cooling chamber should also be cooled to higher temperatures, especially during the freezing and thawing. Therefore, an electrically operated heater that heats the outgassing nitrogen to the desired temperature is arranged in the cooling agent supply line between the cooling agent storage container and the cooling chamber.

Furthermore, the known cooling equipment comprises a control device that measures  
25 the temperature of the cooling agent introduced into the cooling chamber as a control variable and adjusts the heating performance of the heater arranged in the cooling agent supply line as a manipulated variable in order to achieve the desired temperature course in time during freezing and thawing. Thus, the control device  
30 controls only a single heater and evaluates only a single temperature.

However, the previously described, known cooling equipment has the disadvantage of an unsatisfactory control behavior, which expresses itself in an overswinging between the target temperature and the actual temperature and results in a deviation from the desired temperature course in time during freezing and thawing. As a result, 5 the unsatisfactory control behavior of the known cooling equipment can result in damage to the biological samples to be preserved.

The invention therefore has the task of improving the temperature control behavior in the previously described, known cooling equipment.

This task is solved, starting with the initially described, known cooling equipment 10 according to the preamble of Claim 1, by the characterizing features of Claim 1, and, as regards a corresponding operating method, by the features of Claim 23.

The invention comprises the general technical teaching of detecting not only the temperature in the cooling chamber but also at least one other temperature such as, e.g., the temperature of the heated cooling agent supplied to the cooling chamber as 15 control variables.

Furthermore, the invention also comprises the general technical teaching of adjusting at least one further manipulated variable such as, e.g., the heating performance of the evaporator arranged in the cooling agent storage container in addition to the heating performance of the heater arranged in the cooling agent supply line.

20 Therefore, the cooling equipment of the invention preferably has a multiple controller that detects several temperatures as control variables and/or adjusts several heating performances as manipulated variables. The concept of a multiple controller used here is to be understood in a general manner and not limited to a single controller that has several inputs and/or several outputs, but it is also possible that the multiple 25 controller comprises two substantially separate control circuits.

Thus, for example, one control circuit can detect the temperature in the cooling chamber as a control variable and adjust the heating performance of the evaporator as a manipulated variable while another control circuit detects the temperature of the heated cooling agent prior to its introduction into the cooling chamber as a control

variable and adjusts the heating performance of the heater arranged in the cooling agent supply line as a manipulated variable.

If the actual temperature in the cooling chamber is above the target temperature, the heating performance of the evaporator is increased so that more nitrogen outgases and passes into the cooling chamber, which results in a correspondingly greater cooling.

On the other hand, if the actual temperature in the cooling chamber is less than the target temperature, the heating performance of the evaporator is reduced in order that less nitrogen outgases. This down-regulation of the evaporator when the cooling is sufficient also has the advantage that nitrogen is not consumed unnecessarily.

The controlling of the heating performance of the heater arranged in the cooling agent supply line takes place in a similar manner in that this heating performance is increased when the actual temperature of the heated cooling agent is below the target temperature in the cooling chamber. In a corresponding manner, the heating performance of the heater arranged in the cooling agent supply line is reduced if the actual temperature of the heated cooling agent is above the target temperature in the cooling chamber.

In a preferred exemplary embodiment of the invention the measuring of the temperature in the cooling chamber does not take place by a single temperature sensor but rather by several temperature sensors that are preferably arranged in a spatially distributed manner in order to be able to detect local temperature variations within the cooling chamber. The control device can then take into account the formation of local temperature peaks within the cooling chamber by forming an average value and supplying documentation about the actual temperature distribution.

It is furthermore advantageous if at least one temperature sensor has a thermocouple whereas another temperature sensor is designed as a temperature-dependent electrical resistor. Such a combination of different sensor types is appropriate since in this manner the advantages of the different sensor types can be utilized and the disadvantages are avoided. Thus, thermocouples have a good dynamic behavior as temperature sensors but the accuracy is relatively low. On the other hand,

temperature-dependent electrical resistors have a poor dynamic behavior due to their thermal inertia but have great accuracy. Thus, the temperature can be measured very dynamically and very accurately by a combination of these two sensor types.

For example, so-called NTC's (negative temperature coefficients) or PTC's (positive  
5 temperature coefficients) can be used as temperature-dependent electrical resistors.

Furthermore, the cooling equipment of the invention preferably has a storage equipment in order to record the temperature in the cooling chamber and/or the temperature of the heated cooling agent before it enters into the cooling chamber. For example, a commercial PC that is connected via a data interface to the control  
10 device of the cooling equipment according to the invention can be used for this. Furthermore, such a PC can also assume the task of setting the desired temperature courses in time during freezing and thawing.

It is furthermore desirable in the cryopreservation of biological samples to avoid spatial temperature fluctuations within the cooling chamber in order that a defined  
15 freezing or thawing is possible independent of the positioning of the biological sample to be preserved within the cooling chamber. In the preferred exemplary embodiment of the invention, the cooling agent supply line therefore empties via a diffuser into the cooling chamber, the diffuser distributing the cooling agent that is streaming in as uniformly as possible in the cooling chamber. Such a diffuser can consist, e.g., of an  
20 antechamber into which the cooling agent is first introduced, the antechamber being connected over a large area via outlets to the cooling chamber in order to avoid local temperature influences.

In a variant of the invention, the cooling agent supply line empties laterally and preferably only on one side of the cooling chamber into the cooling chamber. This is  
25 advantageous since streams of cooling agents then form inside the cooling chamber that rapidly result in a thorough mixing and a temperature adjustment.

In contrast thereto, in another variant of the invention the cooling agent supply line empties on the top of the cooling chamber into the cooling chamber, which can be particularly advantageous if the cooling chamber is a cooling bell open on the bottom.

The concept of a cooling chamber cited here is therefore not limited to stationary cooling chambers into which the cooled material is introduced, but it is also possible that the cooling chamber is a mobile cooling bell that is placed on the particular cooled material.

- 5 It should also be mentioned that the invention is not limited to nitrogen as cooling agent but it is also possible within the framework of the invention to use other cooling agents such as, e.g., air or helium.

In addition, the invention also comprises a corresponding operating method for such a cooling equipment.

- 10 Other advantageous further developments of the invention are characterized in the dependant claims or are explained in detail in the following together with the description of the preferred exemplary embodiment of the invention using the figures.

Figure 1 shows a schematic view of a cooling equipment in accordance with the invention for the cryopreservation of biological samples.

- 15 Figure 2 shows an alternative exemplary embodiment of a cooling equipment in accordance with the invention.

Figure 3 shows a control-engineering equivalent circuit diagram of the cooling equipment in accordance with the invention.

- 20 Figure 4 shows a temperature course in time in the cooling chamber during the freezing of biological samples.

The cooling equipment shown in figure 1 serves the vitality-preserving cryopreservation of biological samples in which the samples are frozen and thawed in a cooling chamber 1.

- 25 In addition, the cooling equipment has a cooling agent storage container 2 in which liquid nitrogen is present as cooling agent 3, where the cooling agent 3 can be evaporated by an electrically operated evaporator 4.

The cooling agent 3 outgassing into the cooling agent storage container 2 with a temperature close to the boiling point of  $-196^{\circ}\text{C}$  then passes via a cooling agent supply line 5 into the cooling chamber 1, which results in a corresponding cooling.

5 The evaporator 4 has an adjustable heating performance P1 in this instance in order to be able to vary the intensity of the cooling. Thus, a large amount of the cooling agent 3 outgasses at a high heating performance P1 of the evaporator, which results in a corresponding, strong cooling action. On the other hand, less of the cooling agent 3 outgasses at a low heating performance P1 of the evaporator 4 so that the cooling action is less as well.

10 In addition, in order to temper the cooling chamber 1, a heater 6 with an adjustable heating performance P2 is provided, the heater 6 being arranged in the cooling agent supply line 5 and the heating cooling agent 3 outgassing from the cooling agent storage container 2 prior to its entrance into the cooling chamber 1 in order to achieve temperatures above the boiling point of  $-196^{\circ}\text{C}$ , in particular during the  
15 freezing and thawing.

Four temperature sensors 7-10 are provided for monitoring the temperature, the temperature sensor 7 measuring a temperature value T1 that reproduces the temperature of the cooling agent 3 heated by the heater 6 before its entrance into the cooling chamber 1.

20 In contrast thereto, temperature sensors 8-10 measure temperature values T2, T3 and T4 that reproduce the temperature inside the cooling chamber 1 at different points.

The temperature sensors 8-10 are arranged in a spatially distributed manner so that local temperature peaks in the cooling chamber 1 can be compensated by a  
25 formation of an average value.

A control device equipment 11 is provided for temperature control here, that detects temperatures T1-T4 as control variables and adjusts the heating performance P1 of the evaporator 4 and the heating performance P2 of the heater 6 as manipulated variables in order to maintain a desired temperature course in time during freezing  
30 and thawing, where the temperature course can be given by a conventional PC 12

connected to the control device 11 via a data interface. In addition, the PC 12 also records the temperature values T1-T4 measured by the temperature sensors 7-10 and stores them for subsequent evaluation.

It should further be mentioned that the cooling agent supply line 5 does not empty directly into the cooling chamber 1 but rather indirectly via an antechamber 13 in order to avoid spatial temperature variations in the cooling chamber 1. To this end, the antechamber has a diffuser 4 at the transition to the cooling chamber 1 that results in a turbulence of the cooling agent 3 entering into the cooling chamber 1. Furthermore, the discharge cross section of the antechamber 13 is substantially larger at the transition to the cooling chamber 1 than the entrance cross section at the transition from the cooling agent supply line 5 to the antechamber 13, so that the introduction of the cooling agent into the cooling chamber 1 takes place over a relatively large area.

The temperature control behavior of the control device 11 is described in the following using the control-engineering equivalent circuit diagram shown in figure 3.

Thus, the PC 12 constantly sets a target temperature  $T_{\text{TARGET}}$ , that is compared with an actual temperature  $T_{\text{ACTUAL,CHAMBER}}$  by a subtracter 20, the actual temperature  $T_{\text{ACTUAL,CHAMBER}}$  being calculated as the average value of temperatures T2, T3 and T4.

The subtracter 20 calculates a target-actual deviation  $\Delta T_{\text{CHAMBER}}$  from the target temperature  $T_{\text{TARGET}}$  and the actual temperature  $T_{\text{ACTUAL,CHAMBER}}$  and conducts it to a controller 21 that correspondingly adjusts the heating performance P1 of the evaporator 4.

Furthermore, the control-engineering equivalent circuit diagram shows a controlled system 22 that reacts to the heating performance P1 of the evaporator 4 and the heating performance P2 of the heater 6 so that the actual temperature  $T_{\text{ACTUAL,CHAMBER}}$  is adjusted.

In addition to the previously described control circuit for the evaporator 4, the control device 11 has another control circuit for adjusting the heating performance P2 of the heater 6.

Thus, the target temperature  $T_{\text{TARGET}}$  for the temperature inside the cooling chamber 1 is supplied to another subtracter 23 that compares the target temperature  $T_{\text{TARGET}}$  with the actual temperature  $T_1$  of the heated cooling agent. The subtracter 23 calculates a target-actual deviation  $\Delta T_{\text{COOLING AGENT}}$  from the above and supplies it to  
5 another controller 24 that appropriately adjusts the heating performance  $P_2$  of the heater 6, whereupon the controlled system 22 reacts in an appropriate manner so that the actual temperature  $T_1$  is adjusted.

The controller 24 controls the heating performance  $P_2$  of the heater 6 in such a manner in this instance that the actual temperature  $T_1$  of the cooling agent 3 supplied  
10 to the cooling chamber 1 corresponds to the extent possible to the target temperature  $T_{\text{TARGET}}$  in the cooling chamber 1.

The exemplary embodiment shown in figure 2 largely corresponds to the previously described exemplary embodiment shown in figure 1, so that in order to avoid repetitions, reference is made to the previous description for figure 1 and the same  
15 reference signs are used in the following for corresponding structural components, that are characterized by an apostrophe solely in order to distinguish them.

A particularity of this exemplary embodiment consists in the fact that the cooling chamber 1' is designed to be open on its bottom and bell-shaped. Thus, the cooling chamber 1' is mobile in this instance and can therefore be placed on a biological  
20 sample 15' to be frozen, the sample 15' resting on a solid base 16' such as, e.g., a laboratory table. The cooling agent supply line 5' is therefore flexible in this exemplary embodiment in order to make a flexible handling of the cooling chamber 1' possible.

Another difference of this exemplary embodiment from the exemplary embodiment shown in figure 1 is that the cooling agent supply line 5' empties into the cooling chamber 1' at the top of the cooling chamber 1'.  
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Moreover, the cooling equipment in this exemplary embodiment can have another temperature sensor 17' attached in the cooling chamber 1' by a holding arm 18'. The holding arm 18' positions the temperature sensor 17' inside the cooling chamber 1' at  
30 the location at which the sample 15' is located when the cooling chamber 1' is placed on the base 16'. In this manner, the temperature sensor 17' very accurately



measures the local temperature at the location of the sample 15', which makes a very accurate temperature control possible.

Furthermore, a temperature sensor 19' can be arranged directly on the sample 15' or a support carrying the sample 15' in this exemplary embodiment, which makes an even more accurate measuring of the sample temperature possible since local temperature variations in the cooling chamber 1' are not taken into consideration.

The transmission of the temperature measured by the temperature sensor 19' to the control device 11' can take place, e.g., by traditional electrical lines. However, it is basically also possible to transmit the temperature measured by the temperature sensor 19' in a wireless manner to the control device 11'. The mobility and portability of the cooling chamber 1' is not adversely affected by such a wireless transmission. The wireless transmission of the measured temperature can take place, e.g., by a transponder integrated in the temperature sensor 19' or in a sample carrier. There are multiple known possibilities here as regards the transmission type such as, e.g., radio transmission, ultrasonic transmission, optical transmission, in particular infrared transmission, etc.

Finally, figure 4 shows a typical temperature course in time in cooling chamber 1 during the freezing of a biological sample within the framework of cryopreservation. It is apparent from it that several cooling and warming phases are successively passed through during freezing in order to freeze the biological samples while preserving as much vitality as possible.

However, any desired cooling and warming phases are possible within the framework of the invention, where the time of the individual phases and the cooling and warming temperature can be set as desired.

The invention is not limited to the preferred exemplary embodiments previously described, but rather a plurality of variants and modifications are possible that also make use of the concept of the invention and therefore fall within its protective range.

## List of reference signs:

	1, 1'	cooling chamber
	2, 2'	cooling agent storage container
	3, 3'	cooling agent
5	4, 4'	evaporator
	5, 5'	cooling agent supply line
	6, 6'	heater
	7, 7'	temperature sensor
	8, 8'	temperature sensor
10	9, 9'	temperature sensor
	10, 10'	temperature sensor
	11, 11'	control device
	12, 12'	PC
	13, 13'	antechamber
15	14, 14'	diffuser
	15'	sample
	16'	base
	17'	temperature sensor
	18'	holding arm
20	19'	temperature sensor
	20	subtractor
	21	controller
	22	controlled system
	23	subtractor
25	24	controller
	P1, P1'	heating performance of the evaporator
	P2, P2'	heating performance of the heater
	T1, T1'	temperature of the heated cooling agent
	T2-T4, T2', T3'	temperature inside the cooling chamber